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Where are the species limits? Morphology versus genetics in Neotropical chewing lice of the genus *Myrsidea* (Phthiraptera: Menoponidae), with description of three new species

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Abstract

Eleven species of lice of the genus *Myrsidea* Waterston, 1915 (Phthiraptera: Menoponidae) from passerine birds (Passeriformes: Cardinalidae, Fringillidae, Thraupidae) in the Neotropical Region are recorded and discussed. They include three new species with hosts in the family Thraupidae from Paraguay, which are described and illustrated: *Myrsidea flaveolae* new species ex *Sicalis flaveola*, *Myrsidea habiae* new species ex *Habia rubica*, and *Myrsidea sayacae* new species ex *Thraupis sayaca*. Five other previously known *Myrsidea* species are included with new data on intraspecific morphological variability, hosts associations and geographical distribution, as follows: *Myrsidea coronatae*, *M. lightae*, *M. rufi*, *M. seminuda*, and *M. violaceae*. Three further species are recorded at genus level only due to lack of adequate material. A 379 bp portion of the mitochondrial cytochrome oxidase I (*COI*) gene was sequenced in order to assess relative genetic divergence among *Myrsidea* populations. The application of intra- and interspecific sequence divergences as predictor of species boundaries in the taxonomy of this megadiverse genus is discussed.

Key words: *Myrsidea*, Phthiraptera, Amblycera, Menoponidae, lice, new species, new host-louse associations, Passeriformes, Cardinalidae, Fringillidae, Thraupidae, genetic data, intraspecific and interspecific divergences, Paraguay, Honduras, Perú

Introduction

The chewing louse genus *Myrsidea* Waterston, 1915 is the most speciose among Phthiraptera with more than 350 described species (Valim & Weckstein 2013), of which almost 200 species parasitize birds of the Neotropical Region. However, these numbers probably represent only a fragment of the true diversity of *Myrsidea* as discussed by Valim & Weckstein (2013) who estimated that in Brazil alone would be more than 900 additional species of *Myrsidea* awaiting to be collected and described. How to deal with such a huge diversity? The only practical manner is to study the diversity of *Myrsidea* from each host family as a unit, and this practice has been accepted and used by several authors (e.g. Clay 1970; Price & Dalgleish 2007). Accordingly, *Myrsidea* species sharing the same type of male genital sclerite and female bursa copulatrix are frequently found on related hosts, usually from the same family (see Clay 1970). Clay (1968) provided a list of the most important morphological characters as the minimum required for every description of a new *Myrsidea*. Recently, Clay's (1968) system was updated and modified by Valim & Weckstein (2013). However, we often struggle to find the morphological limits that separate species. That is the reason for a combined approach, where morphology is supported with genetic data, as it was done in recent descriptions of *Myrsidea* (e.g. Johnson & Price 2006; Price & Johnson 2006a,b; Price *et al.* 2008a,b,c; Price & Johnson 2009; Valim *et al.* 2011; Valim & Weckstein 2013; Sychra *et al.* 2014a,b). Therefore, we need to resolve taxonomic limits in the genetic data as well, considering that in some cases it is extremely difficult to find species boundaries with DNA data (see Cognato 2006).

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The aim of this paper is to follow up on our previous studies (Sychra *et al.* 2007, 2009; Kounek *et al.* 2011; Kolencik *et al.* 2016) by (1) presenting new data on the distribution of chewing lice of the genus *Myrsidea* found on passerines of the families Cardinalidae, Fringillidae, and Thraupidae in Honduras, Paraguay and Perú, (2) comparing sequences of a portion of the mitochondrial cytochrome oxidase I gene (*COI*) obtained from the species examined against those from other species of *Myrsidea* available in GenBank, (3) discussing the application of intra- and interspecific sequence divergences of *COI* as a predictor of species boundaries within this megadiverse genus, and (4) describing three new species.

Material and methods

Between 2011 and 2014, at various localities in three countries in the Neotropical Region (Honduras, Paraguay and Perú), we used mist nets to trap wild birds to collect their chewing lice. For details referring to study sites, methods of collection and slide-mounting of chewing lice, see Sychra *et al*. (2014b) and Kolencik *et al*. (2016).

Some of the louse samples studied in this paper, belonging to previously described species, differ from their original descriptions or redescriptions by setal counts and dimensions. Only in those cases, we present our data together with those from previous publications.

We used the setal counting system as in Kolencik *et al.* (2016), i.e. (1) the number of metanotal setae does not include the most posterolateral setae; (2) the number of tergal setae on tergite I does not include the postspiracular setae; and (3) the numbers of tergal setae on tergites II–VIII neither include the postspiracular setae nor the short associated setae. Therefore, to avoid misunderstandings, we urge authors to make careful comparison of *Myrsidea* descriptions based on the different systems that include those setae.

In the descriptions, all measurements are in millimeters. Abbreviations for dimensions are: TW, temple width; POW, preocular width; HL, head length at midline; PW, prothorax width; MW, metathorax width; AWIV, abdomen width at level of segment IV; TL, total length; ANW, female anus width; GW, male genitalia width; GSL, genital sac sclerite length. The taxonomy and nomenclature of the birds follow those in Clements *et al.* (2015). The louse species dealt with below are arranged in alphabetical order. All samples, including the type specimens of the new species described in this paper, are deposited in the Moravian Museum, Brno, Czech Republic (MMBC). The new species are attributed to the first two authors only.

Sequences of a 379 bp fragment of the *COI* gene were obtained from: *Myrsidea coronatae* Price & Dalgleish, 2007 (ex *Paroaria capitata* and *Paroaria coronata* from Paraguay), *Myrsidea habiae* new species (ex *Habia rubica* from Paraguay), *Myrsidea lightae* Price, Johnson & Dalgleish, 2008c (ex *Saltator atriceps* from Honduras and ex *Saltator coerulescens* from Paraguay), *Myrsidea sayacae* new species (ex *Thraupis sayaca* from Paraguay), *Myrsidea serini* (Séguy, 1944) (ex *Agelaioides badius* from Paraguay), *Myrsidea violaceae* Price & Dalgleish, 2006 (ex *Euphonia hirundinacea* from Honduras), *Myrsidea* sp. 2 (ex *Trichothraupis melanops* from Paraguay), and *Myrsidea* sp. 3 from *Spinus magellanicus* from Perú.

Methods were those described by Johnson *et al.* (2002). The sequences (GenBank accession numbers (A/N) KY113129–KY113137 and KY249558) were aligned using Geneious 9.1.7 (Kearse *et al.* 2012) and compared to GenBank database using BLAST algorithm (Altschul *et al.* 1990).

In order to assess their genetic divergence, p-distances of sequences from all Neotropical species of *Myrsidea* previously published in the literature and deposited in GenBank (except for *M. nesomimi* where, from 98 available sequences, only single representatives of each of the subspecies *M. nesomimi borealis* Palma & Price, 2010 and *M. nesomimi nesomimi* Palma & Price, 2010 were selected in order to keep the analyses presentable; see Table 1) were computed in Geneious 9.1.7 (Kearse *et al.* 2012), and the information is summarized in Table 3 together with comparable data from other publications. For phylogenetic analysis, the same set of sequences from all Neotropical species of *Myrsidea* (Table 1) and additional sequences of non-Neotropical *Myrsidea* closest to our sequences (Table 2) were used. Phylogenetic analysis was performed on resulting alignment of 379 bp using the maximum likelihood (ML) method based on the GTR+G+I model and conducted by PhyML 2.2.0 plugin in Geneious 9.1.7 (Guindon & Gascuel 2003; Kearse *et al.* 2012) with the GTR+G+I model and parameters estimated from the data; nodal supports were generated with 1,000 bootstrap replicates. The resulting tree with the best likelihood score was chosen, including *Dennyus hirundinis* as an outgroup taxon for rooting (Fig. 10). The tree was visualised using TreeGraph 2.10.1 (Stöver & Müller 2010).

Results

A total of 225 host individuals of 32 bird species were examined. Ninety-nine birds (44%) of 13 species were parasitized with 784 lice (mean intensity = 7.9 lice per bird) belonging to eleven species of *Myrsidea* (Table 4). Three new species were recognized, which are described below. From a total of 13 host-louse associations, nine were found to be new (Table 4). Prevalence of *Myrsidea* species ranged between 29% and 100%. Ranges of mean intensities and mean abundances were 2–24 and 0.7–15.8 respectively. In 77% of parasitized birds (n = 99) the rate of infestation was very low (1–10 lice per bird). Infestations of 11–20 and 21–30 lice per bird were found in 16 (16%) and 5 birds (5%), respectively. The highest numbers of lice per host were found on one *Saltator coerulescens* and one *Paroaria coronata* that harboured 36 and 38 individuals, respectively. The overall sex ratio of lice was equal (168 males versus 153 females; $\chi^2 = 0.7$, $P > 0.05$). The overall age ratio of lice was immatures-biased (321 adults versus 453 immatures; $\chi^2 = 22.5$, $P < 0.01$).

No *Myrsidea* were found on: *Sporophila corvina* (3 birds examined), *Sporophila torqueola* (2), *Tiaris olivaceus* (1) and *Cyanocompsa cyanoides* (3) from Honduras; *Coryphospingus cucullatus* (9), *Poospiza melanoleuca* (11), *Pyrrhocoma ruficeps* (1), *Saltator similis* (2), *Sporophila angolensis* (2), *Volatinia jacarina* (1) and *Cyanocompsa brissonii* (2) from Paraguay; *Diglossa sittoides* (3), *Ramphocelus carbo* (1), *Sicalis luteola* (2), *Sporophila angolensis* (3), *Sporophila telasco* (2), *Tachyphonus luctuosus* (1), *Tiaris obscurus* (3) and *Pheucticus chrysogaster* (5) from Perú.

TABLE 1. GenBank accession numbers of the *COI* sequences of Neotropical *Myrsidea* included in the analyses of genetic diversity in this study

Organism	Accession number (A/N)	Organism	Accession number (A/N)
Myrsidea aurantiirostris	KF048125	*Myrsidea meyi*	JN638821
Myrsidea bessae	EU289213, EU289214	*Myrsidea mitrospingi*	KF048121
Myrsidea brasiliensis	KF048122	*Myrsidea nesomimi borealis*	JF734295
Myrsidea cacioppoi	KF048114	*Myrsidea nesomimi nesomimi*	JF734223
Myrsidea castroae	JN638822	*Myrsidea ochrolaemi*	JN638820
Myrsidea cecilae	KF048126	*Myrsidea oleaginei*	KF048108, KF614514
Myrsidea cicchinoi	FJ171288	*Myrsidea olivacei*	FJ171272
Myrsidea cinnamomei	FJ171286, KF048110	*Myrsidea pagei*	FJ171287
Myrsidea circumsternata	KF048105	*Myrsidea patersoni*	GQ454448
Myrsidea cnemotriccola	KF048124	*Myrsidea rodriguesae*	KF048113
Myrsidea cruikshanki	GQ454449	*Myrsidea rozsai*	FJ171282
Myrsidea diffusa	KF048127	*Myrsidea saviti*	KF048106
Myrsidea elaeniae	KF048117	*Myrsidea seminuda*	FJ171275, KF048116
Myrsidea faccioae	KF048111	*Myrsidea simplex*	FJ171276
Myrsidea fasciata	KF614515	*Myrsidea* sp.	FJ171280
Myrsidea fusca	FJ171267	*Myrsidea* sp.	KF048118
Myrsidea icterocephalae	KF048103	*Myrsidea* sp.	KF048128
Myrsidea isacantha	KF048115	*Myrsidea* sp.	KF048123
Myrsidea johnklickai	EU289212	*Myrsidea* sp.	KF048107
Myrsidea klickai	EU650229	*Myrsidea* sp.	KF048119
Myrsidea laciniaesternata	AF545732	*Myrsidea* sp.	FJ171281
Myrsidea lampropsaricola	KF048104	*Myrsidea* sp.	FJ171290
Myrsidea lanei	KF048120	*Myrsidea* sp.	FJ171291
Myrsidea lathrotriccola	KF048112	*Myrsidea spellmani*	EU650228
Myrsidea lightae	EU289211	*Myrsidea valimi*	GQ454450
Myrsidea melancholici	KF048109	*Myrsidea waterstoni*	FJ171278

TABLE 2. GenBank accession numbers of additional *COI* sequences included in the phylogenetic analysis of this study

Organism	Accession number (A/N)
Myrsidea textoris	KF768813
Myrsidea textoris	KF768814
Myrsidea textoris	KF768815
Myrsidea sp.	DQ887256
Myrsidea sp.	FJ171289

TABLE 3. Intra- and interspecific percent nucleotide differences for chewing lice of the genus *Myrsidea*.

Interspecific differences	Intraspecific differences	No. sequences[1]	Host families	Region	References
13.1–22.1%	0.5–2.3%	7 / 7	Pycnonotidae	Afrotropical	Johnson & Price (2006)
10.7–21.1%		4 / 13	Bernieridae	Afrotropical (Madagascar)	Price & Johnson (2006a)
20.6%		2 / 0	Philepittidae	Afrotropical (Madagascar)	Price & Johnson (2006b)
>12.4%	5.0%	2 / ?	Troglodytidae	Neotropical	Price *et al.* (2008a)
>17–21.6%		2 / ?	Cardinalidae	Neotropical	Price *et al.* (2008b)
12.1%		2 / 16	Thamnophilidae	Neotropical	Price *et al.* (2008c)
>14.5%		5 / ?	Thraupidae	Neotropical	Price & Johnson (2009)
up to 26.7%	0–0.9% 7.4–9.2%	34 / ?	Turdidae + 13 families	Nearctic, Neotropical, Afrotropical	Bueter *et al.* (2009)
	0.1–0.6% 1.3–5.1%	98 / 0	Mimidae	Neotropical (Galápagos Is)	Štefka *et al.* (2011)
>17%		5 / 32	Cotingidae, Emberizidae, Furnariidae, Tyrannidae	Neotropical	Valim *et al.* (2011)
	0.8%	26 / 24	Formicariidae, Icteridae, Thraupidae, Troglodytidae, Tyrannidae	Neotropical	Valim & Weckstein (2013)
17.5%	5.1%	2 / 50	Troglodytidae	Neotropical	Sychra *et al.* (2014b)
14.3–25.9%	0–1.1%	10 / 24	Muscicapidae, Ploceidae, Pycnonotidae, Turdidae	Afrotropical	Sychra *et al.* (2014a)
12–27.3% (mean 20.7%)	0.3–1.3% 5.7–10.5%	32 / ?	Estrildidae, Monarchidae, Pycnonotidae, Turdidae	Afrotropical	Light *et al.* (2016)
>15% (mean 22.2%)		1 / ?	Ploceidae	Afrotropical	Takano *et al.* (2017)
11.7–34.3% (mean 21.8%)	0.3–8.2% 11.1–11.4%	10 / 61	Cardinalidae, Fringillidae, Thraupidae	Neotropical	present study

[1] = number of sequences firstly published in cited references / number of previously published sequences used from GenBank for comparison

Analysis of the Neotropical *Myrsidea COI* sequences obtained from GenBank (Table 1) revealed that interspecific genetic p-distances ranged from 11.7% (between *M. laciniaesternata* Eichler, 1956 and *Myrsidea* sp. ex *Habia fuscator*) to 34.3% (between *M. circumsternata* Valim & Weckstein, 2013 and *Myrsidea* sp. ex *Tangara dowii*).

A total of 1540 comparisons were evaluated for 56 sequences belonging to 52 taxa (Table 1). Five of them with p-distances ranging from 0.8% (e.g. *M. cinnamomei* Dalgleish & Price, 2005) to 5.8% (e.g. *M. oleaginei* Price, Hellenthal & Dalgleish, 2005) were regarded as conspecific *Myrsidea*. Another comparison was made for 1535 interspecific couplets and 99% of them showed genetic p-distances well over 15%. According to these data, we propose that the cut-off limit of interspecific genetic diversity among Neotropical *Myrsidea* species can be tentatively set at about 12% divergence. Some exceptions to these results are discussed in the Discussion below.

Phylogenetic relationships among *Myrsidea* sequences obtained during this study and other Neotropical—and close non-Neotropical species—*Myrsidea* sequences are presented in Fig. 10.

TABLE 4. List of hosts, localities, species, and specimens of *Myrsidea* recorded in this study.

Host family & species	P	E	*Myrsidea* species	♂	♀	N	Country, locality
Cardinalidae							
Habia rubica	6	7	***Myrsidea habiae*	10	15	33	Paraguay, San Rafael NP
Fringillidae							
Euphonia hirundinacea	3	3	*Myrsidea violaceae*	0	1	10	Honduras, Atlántida
Spinus magellanicus	4	14	*Myrsidea* sp. 3	4	4	6	Perú, Cascay, Huanuco
Thraupidae							
Paroaria capitata	13	21	*Myrsidea coronatae*	16	11	42	Paraguay, Los Tres Gigantes BS
Paroaria coronata	12	13	*Myrsidea coronatae*	12	21	68	Paraguay, Teniente Agripino Enciso NP
Paroaria coronata	28	44	*Myrsidea coronatae*	68	40	119	Paraguay, Los Tres Gigantes BS
Saltator atriceps	3	3	***Myrsidea lightae*	6	2	12	Honduras, Atlántida
Saltator coerulescens	8	8	***Myrsidea lightae*	19	27	80	Paraguay, Los Tres Gigantes BS
§*Sicalis flaveola*	1	3	***Myrsidea flaveolae*	1	1	0	Paraguay, Teniente Agripino Enciso NP
§*Sporophila nigricollis*	1	9	*Myrsidea* sp. 3	1	0	0	Perú, Cascay, Huanuco
§*Tachyphonus coronatus*	2	5	***Myrsidea rufi*	2	18	28	Paraguay, San Rafael NP
Thraupis episcopus	14	19	*Myrsidea seminuda*	13	18	37	Perú, Cascay, Huanuco
Thraupis sayaca	2	3	*Myrsidea sayacae*	2	10	18	Paraguay, Los Tres Gigantes BS
§*Tiaris fuliginosus*	1	3	***Myrsidea* sp. 1	1	0	0	Paraguay, San Rafael NP
§*Trichothraupis melanops*	1	13	***Myrsidea* sp. 2	1	0	7	Paraguay, San Rafael NP

P = number of birds parasitized. **E** = number of birds examined. ** = new host-louse association. § = first record of a louse from this host.

Systematics

Phthiraptera Haeckel, 1896: 703.

Amblycera Kellogg, 1896: 68.

Menoponidae Mjöberg, 1910: 26.

Myrsidea Waterston, 1915: 12.

Myrsidea coronatae Price & Dalgleish, 2007

Myrsidea coronatae Price & Dalgleish, 2007: 5, figs 13–15.
Myrsidea coronatae Price & Dalgleish, 2007. Mascarenhas (2008: 62).
Myrsidea coronae [sic] Price & Dalgleish, 2007. Sychra *et al.* (2009: 501).

Type host. *Paroaria coronata* (J.F. Miller, 1776)—Red-crested cardinal (Thraupidae).

Type locality. Kaena Point, Oahu, Hawaii, U.S.A.

Material examined. Ex *Paroaria coronata*: 4♀, 4♂, Teniente Agripino Enciso National Park, Paraguay (21°12'S, 61°39'W), 31 Aug.–2 Sep. 2012; 68♀, 40♂, Los Tres Gigantes Biological Station, Paraguayan Pantanal, Paraguay (20°04'S, 50°09'W), 6–9 Sep. 2012, I. Literak.

Ex *Paroaria capitata* (d'Orbigny & Lafresnaye, 1837)—Yellow-billed cardinal (Thraupidae): 2♀, 2♂, Los Tres Gigantes Biological Station, Paraguayan Pantanal, Paraguay (20°04'S, 50°09'W), 6–9 Sep. 2012, I. Literak.

Remarks. *Paroaria coronata* is distributed over Brazil, Uruguay, Argentina, Paraguay and Bolivia, but it was also introduced to the Hawaiian Islands around 1930 (del Hoyo *et al.* 2011). *Myrsidea coronatae* was originally described from Hawaii by Price & Dalgleish (2007), but it had already been reported from the same host as "*Myrsidea incerta* (Kellogg, 1896)" from Honolulu by Alicata *et al.* (1948). We are confident that this is a wrong identification, because *M. incerta* is parasitic on *Catharus mininus* (Lafresnaye) and *C. ustulatus* (Nuttall) in the family Turdidae (Clay 1966). Furthermore, *Myrsidea coronatae* and *M. incerta* differ by the median gap in tergal setal rows, absent in *M. coronatae*, and the shape of the genital sac sclerite in males. *Myrsidea coronatae* was first reported from mainland South America by Mascarenhas (2008) in Brazil. Later, it was recorded from *Paroaria capitata* in Brazil by Sychra *et al.* (2009). Our samples are the first record of this louse from Paraguay from both hosts.

A portion of *COI* gene was sequenced from specimens of *Myrsidea* from *Paroaria coronata* and *P. capitata* from Paraguay (GenBank accession numbers KY113131 and KY113132). The observed divergence among these samples was 0.3%, justifying their conspecific status. Furthermore, their divergence from other sequences of Neotropical *Myrsidea* (Table 1) exceeded 19.2% in all cases, the closest being *Myrsidea pagei* Price & Johnson, 2009 (A/N FJ171287). *Myrsidea coronatae* is morphologically very close to *M. castroae* Valim, Price & Johnson, 2011, but the net average p-distance between these two species is 19.8%.

Our specimens differ from the original description of *Myrsidea coronatae* by setal counts and dimensions, as follows (data from Price & Dalgleish 2007 are in parentheses):

Female (n = 6). Metanotum posterior margin straight with 6–9 (7–8) setae. Metasternal plate with 6–7 (6) setae. Tergal setae: I, 8–10 (11–13); II, 9–12 (11–13); III, 14–16 (18–24); IV, 18–22 (22–24); V, 18–22 (22–24); VI, 18–20 (21–22); VII, 14–18 (17–20); VIII, 7–10 (10). Sternal setae: II, 14–17 (15–18) marginal setae between asters, 10–13 (14–15) anterior; IV, 34–43 (36–44); VII, 15–18 (16–18). Dimensions: TW, 0.46–0.48 (0.47–0.49); HL, 0.31–0.33 (0.32–0.33); PW, 0.29–0.31 (0.32–0.33); MW, 0.45–0.47 (0.49–0.51); AWIV, 0.61–0.67 (0.66–0.70); TL, 1.50–1.58 (1.61–1.66).

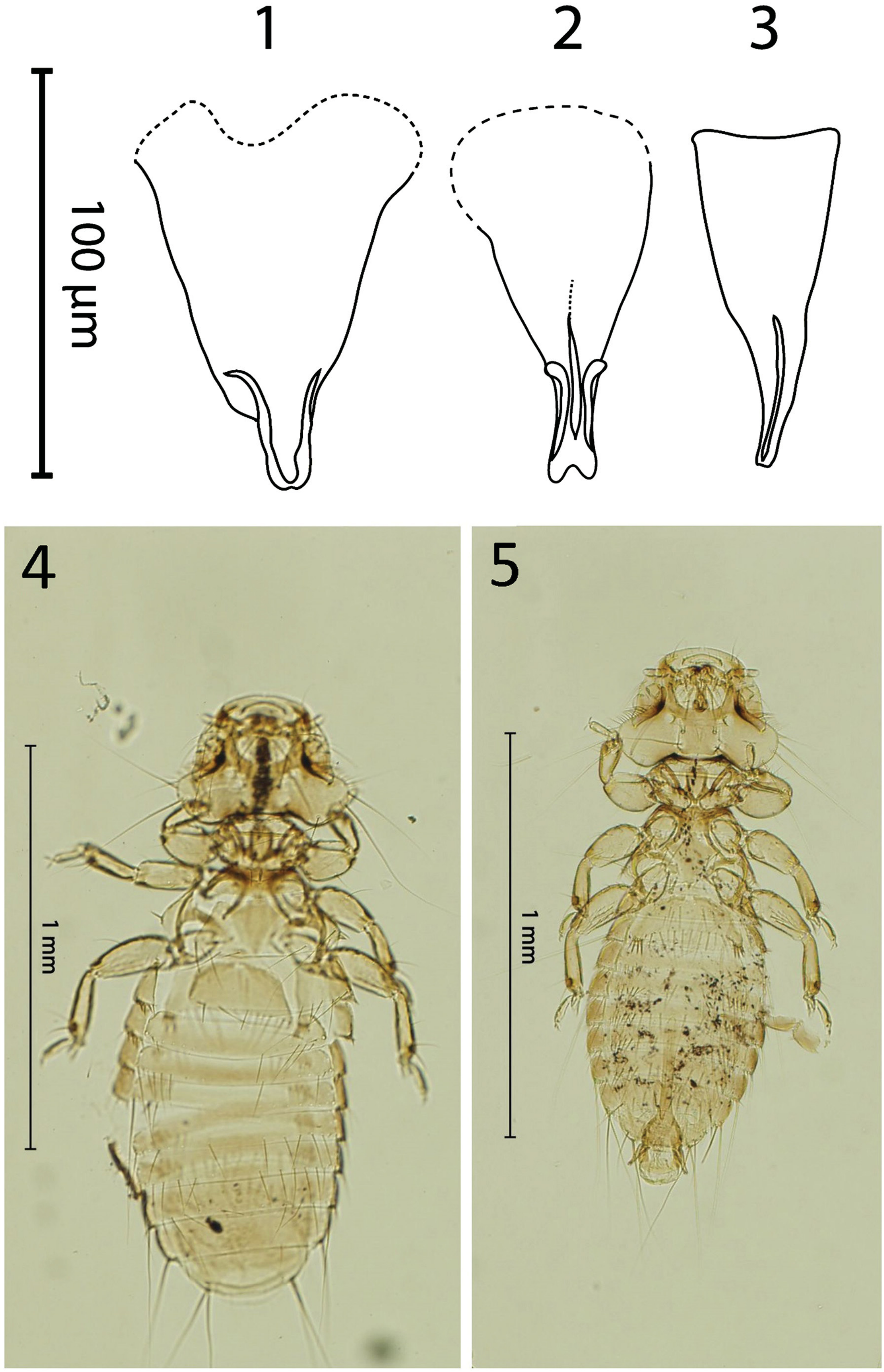
Male (n = 6). Metanotum with 6–8 (7–8) setae. Tergal setae: I, 11–12 (14–16); II, 11–15 (15–18); III, 14–17 (18–24); IV, 16–20 (18–24); V, 17–20 (18–24); VI, 16–20 (18–24); VII, 14–17 (16–22); VIII, 9–11 (11–13). Sternal setae: II, 11–15 (13–16) marginal setae between asters, 7–11 (9–15) anterior; III, 23–30 (29–36); IV, 29–35 (33–39); V, 32–37 (33–39); VI, 26–31 (33–39); VII, 15–20 (22–23). Dimensions: TW, 0.42–0.44 (0.43–0.44); PW, 0.27 (0.29–0.30); MW, 0.37–0.38 (0.38–0.41); TL, 1.27–1.35 (1.29–1.35).

Myrsidea flaveolae Kolencik & Sychra, new species
(Figs 1, 4–5)

Type host. *Sicalis flaveola* (Linnaeus, 1766)—Saffron finch (Thraupidae).

Type locality. Teniente Agripino Enciso National Park, Paraguay (21°12'S, 61°39'W).

Diagnosis. The male genital sac sclerite (Fig. 1) is of the same type of as that of *Myrsidea spizae* (Price & Dalgleish, 2006: fig. 29), which is unique among species of *Myrsidea* from the Thraupidae, but the male of *M. flaveolae* is easily distinguished by having more setae on all tergites (total number of setae on all tergites is 114 against 49–57 in *M. spizae*). The female of *M. flaveolae* is also very similar to that of *M. spizae* in dimensions and number of sternal setae, but differ by the following combination of characters: (1) slightly concave tergites I–III; (2) more setae on tergites I, II, VI–VIII (total number of setae on these five tergites is 50 against 27–38 in *M. spizae*) and (3) postspiracular seta VI as long as I, against postspiracular seta VI much shorter than I in *M. spizae*.



FIGURES 1–5. *Myrsidea* new species. **Figs 1–3,** male genital sac sclerites: **1**, *Myrsidea flaveolae* **n. sp.**; **2**, *Myrsidea habiae* **n. sp.**; **3**, *Myrsidea sayacae* **n. sp. Figs 4–5,** habitus: **4**, *Myrsidea flaveolae* female; **5**, *Myrsidea flaveolae* male.

Description. Female (n = 1). As in Fig. 4. Hypopharyngeal sclerites fully developed. Length of dorsal head seta (DHS) 10, 0.075; DHS 11, 0.100; ratio DHS 10/11, 0.75. Labial setae 5 (*ls5*) 0.06 long, latero-ventral fringe with 12 setae. Gula with 5 setae on each side. Pronotum with 6 setae on posterior margin and 3 short spiniform setae at each lateral corner. First tibia with 4 outer ventro-lateral and 7 dorso-lateral setae. Metanotum not enlarged, with 8 marginal setae; metasternal plate with 6 setae; metapleurites with 2–3 short strong spiniform setae. Femur III with 20–21 setae in ventral setal brush. Tergites not enlarged with medioposterior margins slightly concave on tergites I–III. Abdominal segments with well-defined median gap in each row of tergal setae. Tergal setae: I, 11; II, 12; III, 11; IV, 11; V, 10; VI, 11; VII, 9; VIII, 7. Postspiracular setae very long on II, IV, VII and VIII (0.40–0.45); long on I and VI (0.30–0.31); and short on III and V (0.24). Inner posterior seta of last tergum with length 0.06; length of short lateral marginal seta of last segment, 0.03. Pleural setae: I, 5–6; II, 6; III, 5–6; IV, 5–6; V, 5–6; VI, 5; VII, 4; VIII, 3. Pleurites I–II with only short spine-like setae; pleurites III–VII with 1–2 slender and longer setae on inner ventral side; without anterior setae. Anterior margin of sternal plate II with a medial notch. Sternal setae: I, 0; II, 4 in each aster, aster setae length: s1, 0.04; s2, 0.04; s3, 0.03; s4, 0.02; with 16 marginal setae between asters, 9 medioanterior; III, 23; IV, 36; V, 36; VI, 29; VII, 20; VIII–IX, 14; and 9 setae on deeply serrated vulval margin; without medioanterior setae on sternites. Anal fringe formed by 35 dosral and 28 ventral setae. Dimensions: TW, 0.43; POW, 0.34; HL, 0.30; PW, 0.30; MW, 0.45; AW, 0.58; ANW, 0.19; TL, 1.50.

Male (n = 1). As in Fig. 5. Length of DHS 10, 0.065; DHS 11, 0.090; ratio DHS 10/11, 0.72. *Ls5* 0.08 long, latero-ventral fringe with 10–11 setae. Gula with 4 setae on each side. Pronotum with 6 setae on posterior margin and 3 short spiniform setae at each lateral corner. First tibia with 4 outer ventro-lateral and 6 dorso-lateral setae. Metanotum not enlarged with 10 marginal setae; metasternal plate with 6 setae; metapleurites with 2 short spiniform strong setae. Femur III with 16–17 setae in ventral setal brush. Abdominal segments with continuous row of tergal setae across each segment. Tergal setae: I, 12; II, 13; III, 16; IV, 15; V, 17; VI, 17; VII, 14; VIII, 10. Postspiracular setae very long on II, IV, VII and VIII (0.40–0.45); long on I (0.25); and short on III, V and VI (0.13–0.14). Length of inner posterior seta of last tergum, 0.06; short lateral marginal seta of last segment, 0.02. Pleural setae: I, 4–5; II, 5; III, 6; IV, 6; V, 5; VI, 5; VII, 4–5; VIII, 3. Pleurite I with only short spine-like setae; pleurites II–VII with 1–3 slender and longer setae on inner ventral side; without anterior setae. Anterior margin of sternal plate II with a medial notch. Sternal setae: I, 0; II, 4 in each aster, aster setae length: s1, 0.03–0.04; s2, 0.03; s3, 0.03; s4, 0.02–0.03; with 14 marginal setae between asters, 10 medioanterior; III, 20; IV, 30; V, 33; VI, 32; VII, 19; VIII, 13; remainder of plate, 11; and with 4 setae posteriorly; with 2 medioanterior setae on sternite VIII. With 8 internal anal setae. Genital sac sclerite as in Fig. 1. Dimensions: TW, 0.40; POW, 0.32; HL, 0.28; PW, 0.24; MW, 0.33; AW, 0.44; GW, 0.10; GSL, 0.10; TL, 1.24.

Etymology. This species epithet is a noun in apposition derived from the species name of the type host.

Type material. Ex *Sicalis flaveola*: Holotype ♀, Teniente Agripino Enciso National Park (21°12'S, 61°39'W), 3 Sep. 2012, I. Literak. Paratype: 1♂ with same data as the holotype (MMBC—O. Sychra PG01).

Remarks. This is the first chewing louse known from *Sicalis flaveola*.

Myrsidea habiae **Kolencik & Sychra, new species**
(Figs 2, 6–7)

Type host. *Habia rubica* (Vieillot, 1817)—Red-crowned ant-tanager (Cardinalidae).

Type locality. San Rafael National Park, Paraguay (26°30'S, 55°47'W).

Diagnosis. Due to the same type of enlarged metanotum in females and same type of genital sac sclerite in males (Fig. 2), this species is very similar to *Myrsidea laciniaesternata* Eichler, 1956 (see Price & Dalgleish 2006: figs 1–4) but it can be separated by (1) the larger dimensions of the head (in females TW, 0.48–0.50 and HL, 0.32–0.33 against TW, 0.46–0.48 and HL, 0.31–0.32 in *M. laciniaesternata*; in males TW, 0.43–0.44 and HL, 0.30–0.32 against TW, 0.41–0.43 and HL, 0.29–0.30 in *M. laciniaesternata*) and (2) fewer setae in females on tergites III–V (total number of setae on these tergites is 42–62 against 63–89 in *M. laciniaesternata*) and in males on tergites I–III (total number of setae on these tergites is 23–30 against 35–51 in *M. laciniaesternata*).

Description. Female (n = 4). As in Fig. 6. Hypopharyngeal sclerites fully developed. Length of DHS 10, 0.035–0.037; DHS 11, 0.100–115; ratio DHS 10/11, 0.30–0.37. *Ls5* 0.06 long, latero-ventral fringe with 10–11 setae. Gula with 3–5 setae on each side. Pronotum with 6–7 setae on posterior margin and 3 short spiniform setae at

each lateral corner. First tibia with 3 outer ventro-lateral and 4 dorso-lateral setae. Metanotum enlarged, with 8 marginal setae; metasternal plate with 6–8 setae; metapleurites with 3–4 short strong spiniform setae. Femur III with 15–17 setae in ventral setal brush. Tergites modified as in *M. laciniaesternata* (see Price & Dalgleish 2006: fig. 4). Abdominal segments with well-defined median gap in each row of tergal setae. Tergal setae: I, 17–20; II, 13–24; III, 13–21; IV, 13–21; V, 16–20; VI, 13–15; VII, 10–12; VIII, 4–6. Postspiracular setae very long on II, IV and VIII (0.41–0.49); long on I and VII (0.22–0.31); and short on III, V and VI (0.14–0.20). Inner posterior seta of last tergum not longer than anal fringe setae with length 0.08–0.09; length of short lateral marginal seta of last segment, 0.05–0.06. Pleural setae: I, 4–6; II, 7–8; III, 7–10; IV, 7–8; V, 5–6; VI, 5; VII, 4–5; VIII, 3. Pleurites I–III with only short spine-like setae; pleurites IV–VII with 1–2 slender and longer setae on inner ventral side. Anterior margin of sternal plate II with a medial notch. Sternal setae: I, 0; II, 4–5 in each aster, aster setae length: s1, 0.08; s2, 0.05–0.06; s3, 0.04–0.05; s4, 0.03–0.04; with 15–18 marginal setae between asters, 4–8 medioanterior; III, 23–26; IV, 25–29; V, 30–38; VI, 29–31; VII, 14–21; VIII–IX, 8–15; and 12–13 setae on deeply serrated vulval margin; without medioanterior setae on sternites III–VII. Anal fringe formed by 28–38 dorsal and 30–37 ventral setae. Dimensions: TW, 0.48–0.50; POW, 0.36–0.38; HL, 0.32–0.33; PW, 0.28–0.29; MW, 0.44–0.50; AW, 0.65–0.70; ANW, 0.20–0.23; TL, 1.53–1.55.

Male (n = 4). As in Fig. 7. Length of DHS 10, 0.030–0.035; DHS 11, 0.100–0.110; ratio DHS 10/11, 0.30–0.35. *Ls5* 0.04–0.06 long, latero-ventral fringe with 9–11 setae. Gula with 4–5 setae on each side. Pronotum with 6 setae on posterior margin and 3 short spiniform setae at each lateral corner. First tibia with 3 outer ventro-lateral and 4 dorso-lateral setae. Metanotum enlarged with 4 marginal setae; metasternal plate with 6–7 setae; metapleurites with 3–4 short spiniform strong setae. Femur III with 12–14 setae in ventral setal brush. Abdominal segments with well-defined median gap in each row of tergal setae. Tergal setae: I, 6–8; II, 8–9; III, 9–13; IV, 11–15; V, 9–13; VI, 7–12; VII, 6–8; VIII, 4–5. Postspiracular setae very long on II, IV and VIII (0.48–0.50); long on I and VII (0.24–0.31); and short on III, V and VI (0.13–0.16). Length of inner posterior seta of last tergum, 0.05–0.07; short lateral marginal seta of last segment, 0.02–0.03. Pleural setae: I, 4–5; II, 6–7; III, 7; IV, 5–6; V, 5–6; VI, 5; VII, 4–5; VIII, 3. Pleurites I–III with only short spine-like setae; pleurites IV–VII with 0–2 slender and longer setae on inner ventral side. Anterior margin of sternal plate II with a medial notch. Sternal setae: I, 0; II, 4 in each aster, aster setae length: s1, 0.08–0.09; s2, 0.05–0.06; s3, 0.05; s4, 0.03–0.04; with 12–16 marginal setae between asters, 4–6 medioanterior; III, 18–20; IV, 20–22; V, 23–26; VI, 23–25; VII, 14–17; VIII, 6; remainder of plate, 6–8; with 3 setae posteriorly. With 8 internal anal setae. Genital sac sclerite as in Fig. 2. Dimensions: TW, 0.43–0.44; POW, 0.31–0.33; HL, 0.30–0.32; PW, 0.26–0.28; MW, 0.37–0.39; AW, 0.46–0.47; GW, 0.110–0.115; GSL, 0.09; TL, 1.23–1.30.

Etymology. This species epithet is a noun in apposition derived from the generic name of the type host.

Type material. Ex *Habia rubica*: Holotype ♀, San Rafael National Park, Paraguay (26°30'S, 55°47'W), 18–22 Aug. 2012, I. Literak. Paratypes: 3♀, 4♂ with the same data as holotype (MMBC—O. Sychra PG02–05).

Remarks. This is the first record of a chewing louse from *Habia rubica* in Paraguay. A portion of *COI* gene was sequenced from our specimens of *Myrsidea habiae* (GenBank accession number KY113133). Comparing our sequence with other known sequences of Neotropical *Myrsidea*, the closest was that of *M. simplex* Ansari, 1956 (A/N FJ171276) with a sequence divergence of 17.7%. Furthermore, comparing our sequence of *M. habiae* against all known sequences of *Myrsidea*, the closest was that of *Myrsidea* sp. ex *Seiurus aurocapillus* (A/N FJ171289) from the U.S.A., with a p-distance of 16.1%. These sequence divergences are large enough to confirm *M. habiae* as a separate species.

Myrsidea lightae Price, Johnson & Dalgleish, 2008

Myrsidea lightae Price, Johnson & Dalgleish, 2008c: 2, figs 1–4.
Myrsidea lightae Price, Johnson & Dalgleish, 2008. Kounek *et al.* (2011: 9).

Type host. *Saltator maximus* (Statius Müller, 1776)—Buff-throated saltator (Thraupidae).

Type locality. Serriana del Maje, Panamá.

Material examined. Ex *Saltator atriceps* (Lesson, 1832)—Black-headed saltator (Thraupidae): 4♀, 2♂, Atlántida, Tela, Botanical Garden Lancetilla, Honduras (15°44' N, 87°27' W), 13 Aug. 2014, I. Literak.

Ex *Saltator coerulescens* Vieillot, 1817—Greyish saltator (Thraupidae): 7♀, 7♂, Los Tres Gigantes Biological Station, Paraguayan Pantanal, Paraguay (20°04'S, 50°09'W), 6–9 Aug. 2012, I. Literak.

Remarks. This species was previously described from *Saltator maximus* and *Saltator striatipectus* Lafresnaye, 1847 from Costa Rica, Panamá and Venezuela (Price *et al.* 2008c). Our host records are new host-louse associations for *M. lightae* and the first records of this louse species from Honduras and Paraguay.

A portion of *COI* gene was sequenced from specimens of *Myrsidea lightae* ex *Saltator atriceps* from Honduras and from *S. coerulescens* from Paraguay (GenBank accession numbers KY113134 and KY113135, respectively). Comparing our sequences with those of *M. lightae* from *S. maximus* from Panamá (A/N EU289211), the following sequence divergences were found: between lice from *S. atriceps* and *S. maximus*: 8.2%; between lice from *S. maximus* and *S. coerulescens*: 11.1%, and between lice from *S. atriceps* and *S. coerulescens*: 11.4%. All three divergences fall well under or close to the limit of interspecific variation for *Myrsidea* species as proposed in this paper. We believe that these genetic data together with minimal morphological differences (see below), justify the conspecific status of the *Myrsidea* populations from all the hosts and localities mentioned above.

Our specimens differ from the original description of *Myrsidea lightae* by setal counts and dimensions, as follows (data from Price *et al.* 2008c are in parentheses):

Female ex *Saltator atriceps* (n = 4). Gula with 5–6 (4–5) setae on each side. Length of postspiracular setae: III, 0.34–0.39 (short); VII, 0.30–0.39 (long). Sternal setae: II, 2–6 (6–12) anterior; III, 39–40 (32–35); VI, 40–45 (33–43). Anus with 42–49 (34–40) dorsal and 35–37 (38–46) ventral fringe setae. Dimensions: TW, 0.55–0.58 (0.51–0.56); HL, 0.36–0.37 (0.34–0.36); AWIV, 0.67–0.73 (0.68–0.75); ANW, 0.25–0.26 (0.24); TL, 1.75–1.85 (1.67–1.82).

Male ex *Saltator atriceps* (n = 2). Tergal setae: V, 11–14 (13–18); VIII, 6–10 (8–11). Sternal setae: 4–5 (7–14) anterior; III, 21–34 (24–33); IV, 31–42 (38–46); V, 37–43 (39–47); VIII, 6–12 (7–12). Dimensions: TW, 0.51 (0.46–0.50); HL, 0.32–0.34 (0.28–0.33); MW, 0.44–0.46 (0.41–0.45); GSL, 0.11 (0.09); TL, 1.50–1.53 (1.34–1.47).

Female ex *Saltator coerulescens* (n = 7). Gula with 5–6 (4–5) setae on each side. Metanotum with 14–19 (15–1) setae on posterior margin. Tergal setae: I, 10–16 (11–18); II, 11–17 (13–18); III, 11–16 (13–20); IV, 10–15 (13–18); VI, 11–15 (12–17). Length of postspiracular setae: III, 0.27–0.30 (short); VII, 0.24–0.30 (long). Sternal setae: II, 2–3 (6–12) anterior; III, 31–45 (32–35); IV, 45–58 (42–52); V, 43–57 (40–53); VI, 36–50 (33–43); VII, 14–17 (15–20). Anus with 41–47 (34–40) dorsal and 36–42 (38–46) ventral fringe setae. Dimensions: HL, 0.34–0.37 (0.34–0.36); PW, 0.30–0.34 (0.33–0.35); AWIV, 0.63–0.75 (0.68–0.75); ANW, 0.25–0.28 (0.24); TL, 1.63–1.76 (1.67–1.82).

Male ex *Saltator coerulescens* (n = 7). Tergal setae: II, 11–16 (12–17); VII, 10–14 (11–16). Sternal setae: II, 17–20 (15–19) marginal setae between asters; III, 28–37 (24–33); IV, 37–48 (38–46); VI, 39–46 (24–41); VIII, 11–15 (7–12). Dimensions: PW, 0.27–0.31 (0.30–0.32); MW, 0.39–0.46 (0.41–0.45); GW, 0.12–0.13 (0.13); GSL, 0.09–0.10 (0.09); TL, 1.38–1.53 (1.34–1.47).

Myrsidea rufi Price & Dalgleish, 2006

Myrsidea rufi Price & Dalgleish, 2006: 16, figs 22–23.

Type host. *Tachyphonus rufus* (Boddaert, 1783)—White-lined tanager (Thraupidae).

Type locality. Arima, Simla, Trinidad.

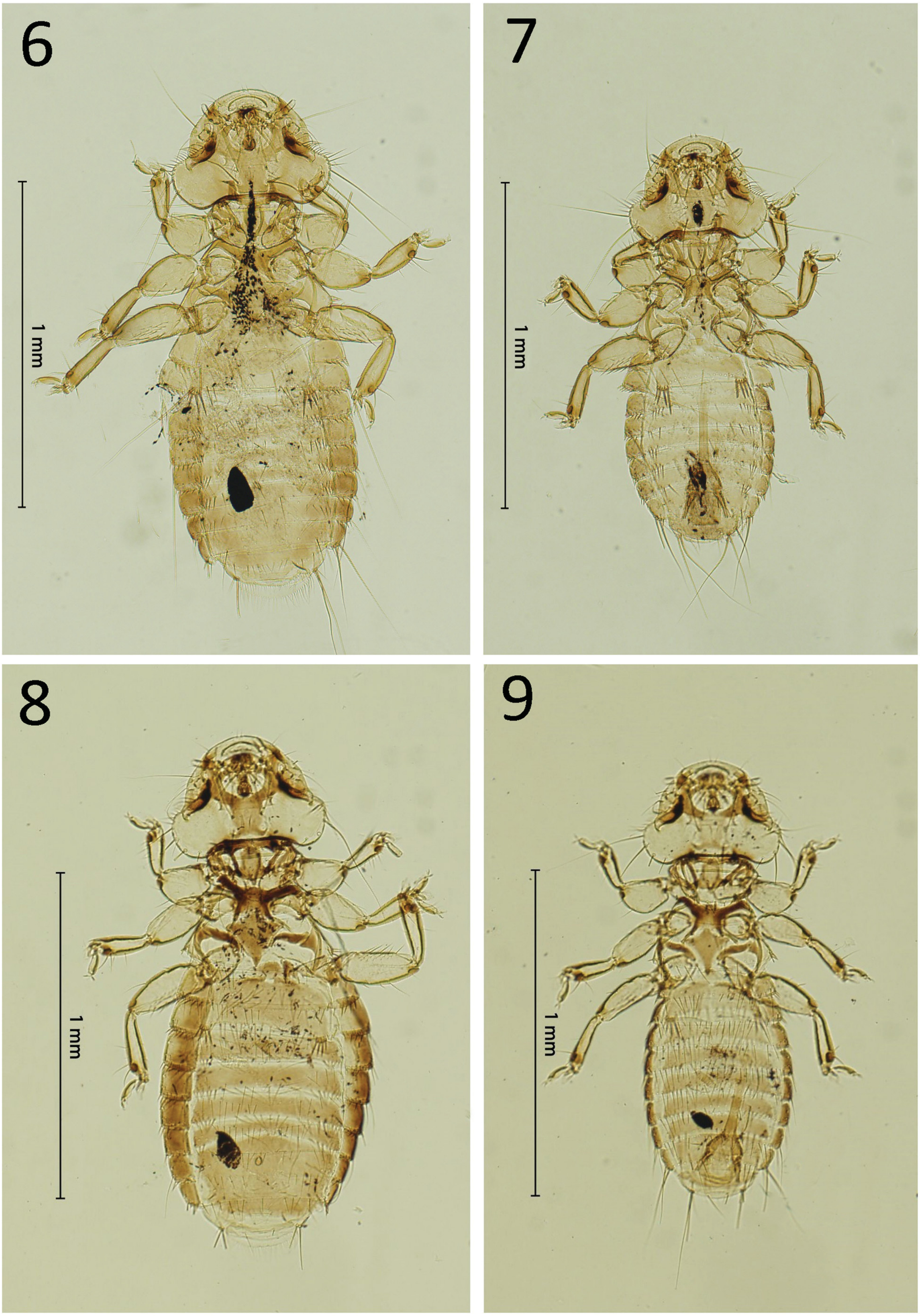
Material examined. Ex *Tachyphonus coronatus* (Vieillot, 1822)—Ruby-crowned tanager (Thraupidae): 1♀, 4♂, San Rafael National Park, Paraguay (26°30'S, 55°47'W), 18 & 22 Aug. 2012, I. Literak.

Remarks. *Myrsidea rufi* was described from Trinidad & Tobago and also found on the type host in Brazil (Silva 2013). This is the first record of *M. rufi* from Paraguay, and of a louse from *Tachyphonus coronatus*.

Our specimens differ from the original description of *Myrsidea rufi* by setal counts and dimensions, as follows (data from Price & Dalgleish 2006 are in parentheses):

Female (n = 1). Metanotum with 12 (8–10) setae on posterior margin. Tergal setae: I, 18 (14–16); II, 17 (9–13); VII, 7 (8–12); VIII, 4 (6–8). Sternal setae: VII, 9 (10–13). Dimensions: TW, 0.42 (0.46–0.49); AWIV, 0.55 (0.59–0.64).

Male (n = 4). Metanotum with 8–10 (6–9) setae on posterior margin. Tergal setae: I, 14–17 (9–14); II, 12–13 (7–12); III, 12–15 (7–12); VI, 8–11 (7–10); VIII, 4 (6–7). Sternal setae: II, 4 setae in each aster, 13–16 marginal setae between asters, 6–8 anterior (together 27–32 vs. 22–26); V, 29–34 (30–35); VI, 25–28 (26–33); VII, 12–15 (13–19); VIII, 11–14 (5–9). Dimensions: MW, 0.34–0.38 (0.38–0.40); AWIV, 0.47–0.48 (0.48–0.53); TL, 1.25–1.34 (1.28–1.34).



FIGURES 6–9. *Myrsidea* new species, habitus. *Myrsidea habiae* **n. sp.**: **6**, female; **7**, male. *Myrsidea sayacae* **n. sp.**: **8**, female; **9**, male.

***Myrsidea sayacae* Kolencik & Sychra, new species**
(Figs 3, 8–9)

«*Myrsidea seminuda* Eichler, 1951»: Sychra *et al.* (2009: 502). Ex *Thraupis sayaca*. Misidentification.

Type host. *Thraupis sayaca* (Linnaeus, 1766)—Sayaca tanager (Thraupidae).

Type locality. Tres Gigantes Biological Station, Paraguayan Pantanal, Paraguay (20°04'S, 50°09'W).

Diagnosis. This species is close to *M. seminuda* Eichler, 1951 in (1) body shape (see figs 13–14 in Price & Dalgleish 2006), (2) dimensions, and (3) the presence of medioanterior setae on tergit III. However, *M. sayacae* is easily distinguished from *M. seminuda* by fewer setae on all tergites: in females, the total number of setae on all tergites is 160–181, against 191–256 in *M. seminuda*; in males it is 162–212 against 208–254 in *M. seminuda*.

Description. Female (n = 3) As in Fig. 8. Hypopharyngeal sclerites fully developed. Length of DHS 10, 0.060–0.067; DHS 11, 0.103–0.108; ratio DHS 10/11, 0.59–0.62. *Ls5* 0.08–0.09 long, latero-ventral fringe with 9–10 setae. Gula with 5 setae on each side. Pronotum with 6 setae on posterior margin and 3 short spiniform setae at each lateral corner. First tibia with 3 outer ventro-lateral and 4 dorso-lateral setae. Metanotum not enlarged, with 11–12 marginal setae; metasternal plate with 6–7 setae; metapleurites with 3 short strong spiniform setae. Femur III with 18–19 setae in ventral setal brush. Tergites not enlarged, all with straight posterior margin. Abdominal segments with continuous row of tergal setae across each segment. Tergal setae: I, 23–26; II, 21–26; III, 16–18; IV, 21–23; V, 25–27; VI, 23–25; VII, 19–22; VIII, 12–14. Postspiracular setae very long on VIII (0.43); long on VI (0.28); and short on I and VII (0.23–0.24). Inner posterior seta of last tergum not longer than anal fringe setae with length 0.07; length of short lateral marginal seta of last segment, 0.04. Pleural setae: I, 6–8; II, 7–10; III, 7–8; IV, 6–7; V, 6–7; VI, 5–6; VII, 4; VIII, 3. Pleurites I–II with only short spine-like setae; pleurites III–VII with 2–3 slender and longer setae. Anterior margin of sternal plate II with a medial notch. Sternal setae: I, 0; II, 4 in each aster, aster setae length: s1, 0.04–0.06; s2, 0.04–0.05; s3, 0.04–0.05; s4, 0.03; with 18–26 marginal setae between asters, 15–21 medioanterior; III, 31–37; IV, 32–34; V, 33–36; VI, 26–27; VII, 14–18; VIII–IX, 11–13; and 9–12 setae on deeply serrated vulval margin; with 5–9 medioanterior setae on sternite III. Anal fringe formed by 36–41 dosral and 33–35 ventral setae. Dimensions: TW, 0.42–0.48; POW, 0.35–0.38; HL, 0.30–0.33; PW, 0.22–0.30; MW, 0.44–0.45; AW, 0.58–0.65; ANW, 0.20–0.22; TL, 1.50–1.55.

Male (n = 8). As in Fig. 9. Length of DHS 10, 0.058–0.075; DHS 11, 0.100–0.110; ratio DHS 10/11, 0.57–0.71. *Ls5* 0.07 long, latero-ventral fringe with 10–11 setae. Gula with 4–5 setae on each side. Pronotum with 6 setae on posterior margin and 3 short spiniform setae at each lateral corner. First tibia with 3 outer ventro-lateral and 4 dorso-lateral setae. Metanotum not enlarged with 8–10 marginal setae; metasternal plate with 6–7 setae; metapleurites with 1–3 short spiniform strong setae. Femur III with 13–17 setae in ventral setal brush. Abdominal segments with continuous row of tergal setae across each segment. Tergal setae: I, 19–23; II, 21–25; III, 22–29; IV, 23–31; V, 22–31; VI, 22–29; VII, 20–25; VIII, 13–19. Postspiracular setae very long on VIII (0.36–0.39); long on II, IV and VII (0.25–0.35); and short on I, III, V and VI (0.13–0.20). Length of inner posterior seta of last tergum, 0.1; short lateral marginal seta of last segment, 0.02. Pleural setae: I, 4–5; II, 6–7; III, 6–7; IV, 6; V, 5; VI, 5; VII, 4; VIII, 3. Pleurites I with only short spine-like setae; pleurites II–VII with 0–4 slender and longer setae on inner ventral side. Anterior margin of sternal plate II with a medial notch. Sternal setae: I, 0; II, 3–4 in each aster, aster setae length: s1, 0.04–0.06; s2, 0.04–0.05; s3, 0.03–0.04; s4, 0.03; with 12–19 marginal setae between asters, 8–16 medioanterior; III, 26–34; IV, 29–34; V, 28–35; VI, 25–30; VII, 16–22; VIII, 8–12; remainder of plate, 9–13; and with 3 setae posteriorly; with 4–9 medioanterior setae on sternit III. With 8–9 internal anal setae. Genital sac sclerite as in Fig. 3. Dimensions: TW, 0.40–0.44; POW, 0.32–0.38; HL, 0.27–0.30; PW, 0.26–0.33; MW, 0.35–0.37; AW, 0.45–0.47; GW, 0.10–0.11; GSL, 0.08–0.09; TL, 1.23–1.35.

Etymology. The species epithet is a noun in apposition derived from the species name of the type host.

Type material. Ex *Thraupis sayaca*: Holotype ♀, Tres Gigantes Biological Station, Paraguayan Pantanal, Paraguay (20°04'S, 50°09'W), 6 Sep. 2012, I. Literak. Paratypes: 2♀, 6♂ with the same data as holotype (MMBC—O. Sychra PG06–09).

Additional material examined (non-types). Ex *Thraupis sayaca,* 1♀, 2♂, Nova Andradina (22˚15'S, 53˚21'W), Mato Grosso do Sul, Brazil, 29–30 Jul. 2006, I. Literak & M. Capek.

Remarks. Sychra *et al.* (2009: 502) identified a sample of *Myrsidea* from *Thraupis sayaca* from Brazil as *M. seminuda*. However, our reexamination of that sample and new material from Paraguay showed that they belong to

a new species. A portion of *COI* gene was sequenced from specimens of *Myrsidea sayacae* (GenBank accession number KY113136). As expected, the closest species among other known sequences of Neotropical *Myrsidea* was that of *M. seminuda* (A/N FJ171275), with a sequence divergence of 14.3%. This divergence is close to the limit of interspecific variation proposed in this paper but, together with their morphological differences, we are confident that *M. sayacae* and *M. seminuda* represent separate species.

Myrsidea seminuda Eichler, 1951

Myrsidea seminuda Eichler, 1951: 53.
Myrsidea seminuda Eichler, 1951: Price *et al.* (2003: 131); Price & Dalgleish (2006: 11, figs 13–14); Kounek *et al.* (2011: 13).

Type host. *Thraupis palmarum* (Wied, 1821)—Palm tanager (Thraupidae).

Type locality. Unknown.

Material examined. Ex *Thraupis episcopus* (Linnaeus, 1766)—Blue-grey tanager (Thraupidae): 6♀, 5♂, Cascay, Huanuco, Perú (9°50'S, 76°08'W), 20–23 Aug. 2011, I. Literak.

Remarks. *Myrsidea seminuda* was redescribed from material collected from the type host and *T. episcopus* in Costa Rica and Trinidad (Price & Dalgleish 2006; Kounek *et al.* 2011). Our material is the first record of this louse from Perú. The record of *M. seminuda* in Sychra *et al.* (2009: 502) from *Thraupis sayaca* from Brazil is a misidentification of *M. sayacae* (see above). The geographic distribution of these *Myrsidea* populations agrees with their separation in two species, with *M. seminuda* recorded in the north-west of the Neotropical Region (Costa Rica, Trinidad, Perú), while *M. sayacae* is found in the south-east of the Region (southern Brazil, Paraguay).

Our specimens differ from the previous descriptions of *M. seminuda* by setal counts and dimensions, as follows (overall data taken from Price & Dalgleish 2006, and Kounek *et al.* 2011 are in parentheses):

Female (n = 6). Gula with 4–7 (3–6) setae on each side. Metanotum with 9–12 (9–12) setae on posterior margin. Tergal setae: III, 22–29 (27–32); V, 30–36 (29–39); VI, 26–33 (25–32). Sternal setae: II, 17–20 marginal setae between asters, 19–26 anterior (together 36–46 vs. 32–44); VI, 26–32 (25–31); VII, 16–23 (15–18). Dimensions: TW, 0.44–0.48 (0.45–0.47); HL, 0.31–0.33 (0.30–0.32); AWIV, 0.55–0.61 (0.56–0.61); ANW, 0.20–0.22 (0.18–0.20); TL, 1.50–1.60 (1.48–1.59).

Male (n = 5). Metanotum with 9–11 (10–11) setae on posterior margin. Tergal setae: III, 29–32 (30–38); IV, 29–36 (30–38); V, 29–34 (30–38); VI, 30–34 (32–35); VIII, 14–17 (16–18). Sternal setae: II, 11–16 marginal setae between asters, 13–20 anterior (together 24–36vs. 25–37); III, 27–40 (30–42); IV, 32–42 (30–41); V, 28–41 (31–38); VI, 28–37 (24–31); VII, 18–26 (17–20); VIII, 10–17 (12–17). Dimensions: TW, 0.40–0.43 (0.41–0.43); PW, 0.26–0.29 (0.27–0.29); TL, 1.27–1.37 (1.24–1.29).

Myrsidea violaceae Price & Dalgleish, 2006

Myrsidea violaceae Price & Dalgleish, 2006: 9.
Myrsidea violaceae Price & Dalgleish, 2006: Sychra *et al.* (2007: 63), Kounek *et al.* (2011: 14).

Type host: *Euphonia violacea* (Linnaeus, 1758)—Violaceous euphonia (Fringillidae).

Type locality. Simla Biological Station, Trinidad.

Material examined. Ex *Euphonia hirundinacea* (Lesson, 1832)—Yellow-throated euphonia (Fringillidae): 1♂, Atlántida, Tela, Botanical Garden Lancetilla, Honduras (15°44' N 87°27' W), 16 Aug. 2014, I. Literak.

Remarks. This species was previously recorded from Trinidad and Costa Rica (Price & Dalgleish 2006; Sychra *et al.* 2007; Kounek *et al.* 2011). This is the first record of *M. violaceae* from Honduras.

A portion of *COI* gene was sequenced from our single specimen of *Myrsidea violaceae* from *Euphonia hirundinacea* (GenBank accession number KY113137). Comparing our sequence with other known sequences of Neotropical *Myrsidea*, the closest was that of *Myrsidea rozsai* Price & Johnson, 2009 from *Euphonia lanniirostris* (A/N FJ171282), with a sequence divergence of 11.9%. This divergence is on the border of the interspecific variation limit for *Myrsidea* species as proposed in this paper. More specimens of *Myrsidea* from the above and other species of *Euphonia* are needed to resolve the taxonomic status of these two nominal species, based on molecular and morphological data.

Our specimen differs from descriptions of *M. violaceae* by setal counts and dimensions, as follows (overall data from Price & Dalgleish 2006, Sychra *et al.* 2007, and Kounek *et al.* 2011 are in parentheses):

Male (n = 1). Tergal setae: VIII, 12 (14–18). Sternal setae: VIII+IX, 15 (7–14). Dimensions: POW, 0.31 (0.29–0.30); TL, 1.23 (1.11–1.21).

Myrsidea sp. 1

Material examined. Ex *Tiaris fuliginosus* (Wied, 1830)—Sooty grassquit (Thraupidae): 1♂, San Rafael National Park, Paraguay (26°30'S, 55°47'W), 22 Aug. 2012, I. Literak.

Remarks. This is the first record of a chewing louse from *Tiaris fuliginosus*. Our male differs morphologically from those of other Neotropical *Myrsidea* by a combination of several important features: (1) presence of median gap in most rows of tergal setae, (2) only 4 metanotal setae, (3) relatively high number of tergal setae, especially VIII with 9 setae, (4) genital sac sclerite without subapical projection on each side, and (5) small dimensions, especially TW, 0.37. This male *Myrsidea* from *Tiaris fuliginosus* probably represents an undescribed species, but having only one male is insufficient for an adequate description of a new species.

Myrsidea sp. 2

Material examined. Ex *Trichothraupis melanops* (Vieillot, 1818)—Black-goggled tanager (Thraupidae): 1♂, San Rafael National Park, Paraguay (26°30'S, 55°47'W), 20 Aug. 2012, I. Literak.

Remarks. This is the first record of a chewing louse from *Trichothraupis melanops*. A portion of *COI* gene was sequenced from our single male specimen from *T. melanops* (GenBank accession number KY249558). Comparing our sequence with other known sequences of Neotropical *Myrsidea*, the closest was *Myrsidea fusca* (Carriker, 1903) (A/N FJ171267) with a sequence divergence of 16.0%. Furthermore, *Myrsidea* sp. 2 is morphologically very close to *M. patersoni* Price & Johnson, 2009 (A/N GQ454448), but the net average p-distance between these two species is 18.5%. These divergences would justify the recognition of our specimen as a separate species. However, having only one male is insufficient for an adequate description of a new species.

Myrsidea sp. 3

Material examined. Ex *Spinus magellanicus* (Vieillot, 1805)—Hooded siskin (Fringillidae): 4♀, 2♂, Cascay, Huanuco, Perú (9°50'S, 76°08'W), 20 & 22 Aug. 2011, I. Literak (MMBC—O. Sychra PE16–19).

Ex *Sporophila nigricollis* (Vieillot, 1823)—Yellow-bellied seedeater (Thraupidae): 1♂, Cascay, Huanuco, Perú (9°50'S, 76°08'W), 21 Aug. 2011, I. Literak (MMBC—O. Sychra PE20).

Remarks. A portion of *COI* gene of *Myrsidea* from *Spinus magellanicus* from Perú was sequenced (GenBank accession number KY113129). Comparing this sequence with those from other species of Neotropical *Myrsidea* with known sequences (Table 1), *Myrsidea* from *S. magellanicus* is highly different from all of them, with p-distance exceeding 18.2% in all cases, and the closest being *Myrsidea seminuda,* A/N FJ171275. The exception is *M. serini* from *Agelaoides badius* from Paraguay (GenBank accession number KY113130). The divergence among our data of *Myrsidea* from *Spinus magellanicus* and that of *M. serini* is 6.6%, indicating that they could be conspecific. Curiously, considering species from other regions, the closest to our sequence of *Myrsidea* from *S. magellanicus* was that of *M. textoris* ex *Ploceus intermedius* and *Ploceus velatus* (A/N KF768813 and KF768815) from South Africa, with p-distance of only 5.3%, followed by *Myrsidea* sp. ex *Vidua macroura* (A/N DQ887256) from Cameroon with 7.7%. This relatively small genetic divergences lead us to leave our sample of *Myrsidea* from *Spinus magellanicus* as undetermined at the species level, at least until we re-evaluate the morphometric characteristics of this and other closely related species.

Discussion

Our analysis of the *COI* sequences of Neotropical *Myrsidea* obtained from GenBank revealed that interspecific genetic p-distances ranged from 11.7% to 34.3%. Most of the evaluated p-distances showed interspecific differences well over 15%. We found only three exceptions with p-distances ranging from 11.7% to 13%: (1) *M. bessae* Price, Johnson & Dalgleish, 2008a versus *M. cinnamomei* with p-distance 13%; (2) *M. klickai* Price, Johnson & Dalgleish, 2008b versus *M. spellmani* Price, Johnson & Dalgleish, 2008b with p-distance 12.1%; and (3) *M. laciniaesternata* versus *Myrsidea* sp. from *Habia fuscator* with p-distance 11.7%. However, in the first two pairs (1 and 2), morphological differences support a clear separation of the species included. The couple given in (3) remains questionable because there are no morphological data to separate them.

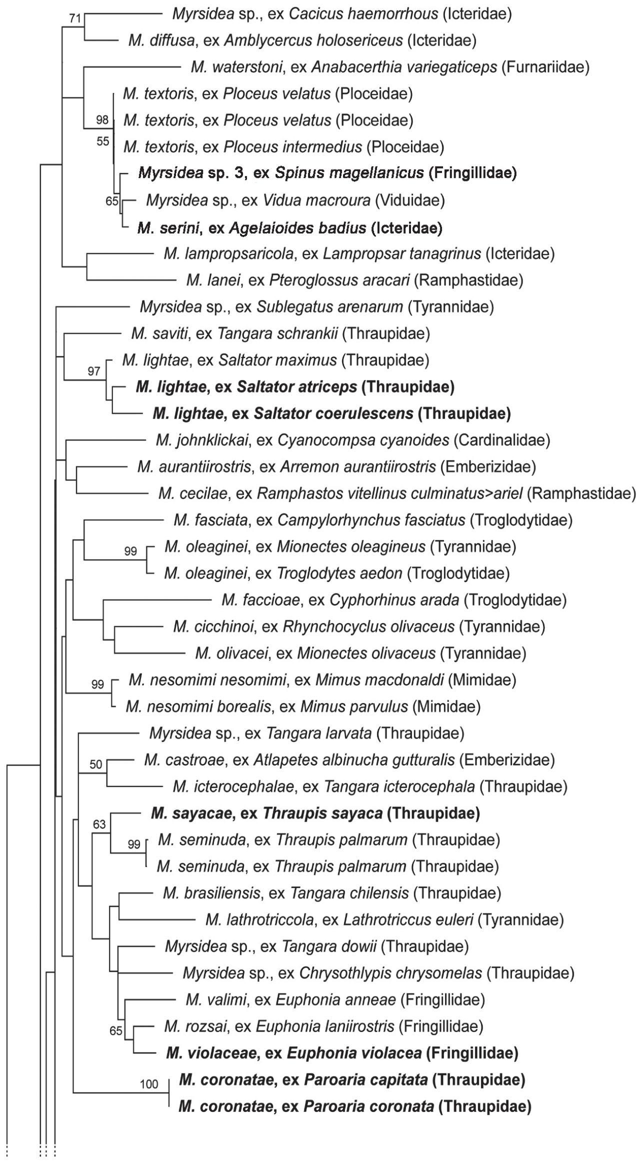


FIGURE 10. Phylogenetic tree of the Neotropical *Myrsidea* species (including additional non-Neotropical *Myrsidea* closest to species examined) based on partial *COI* sequences. The tree was inferred using the maximum likelihood method based on the GTR+G+I model. The tree with the highest log likelihood is shown. Bootstrap support is shown next to the branches (values <50 % not shown). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Species of *Myrsidea* discussed in the present paper are in **bold** type.

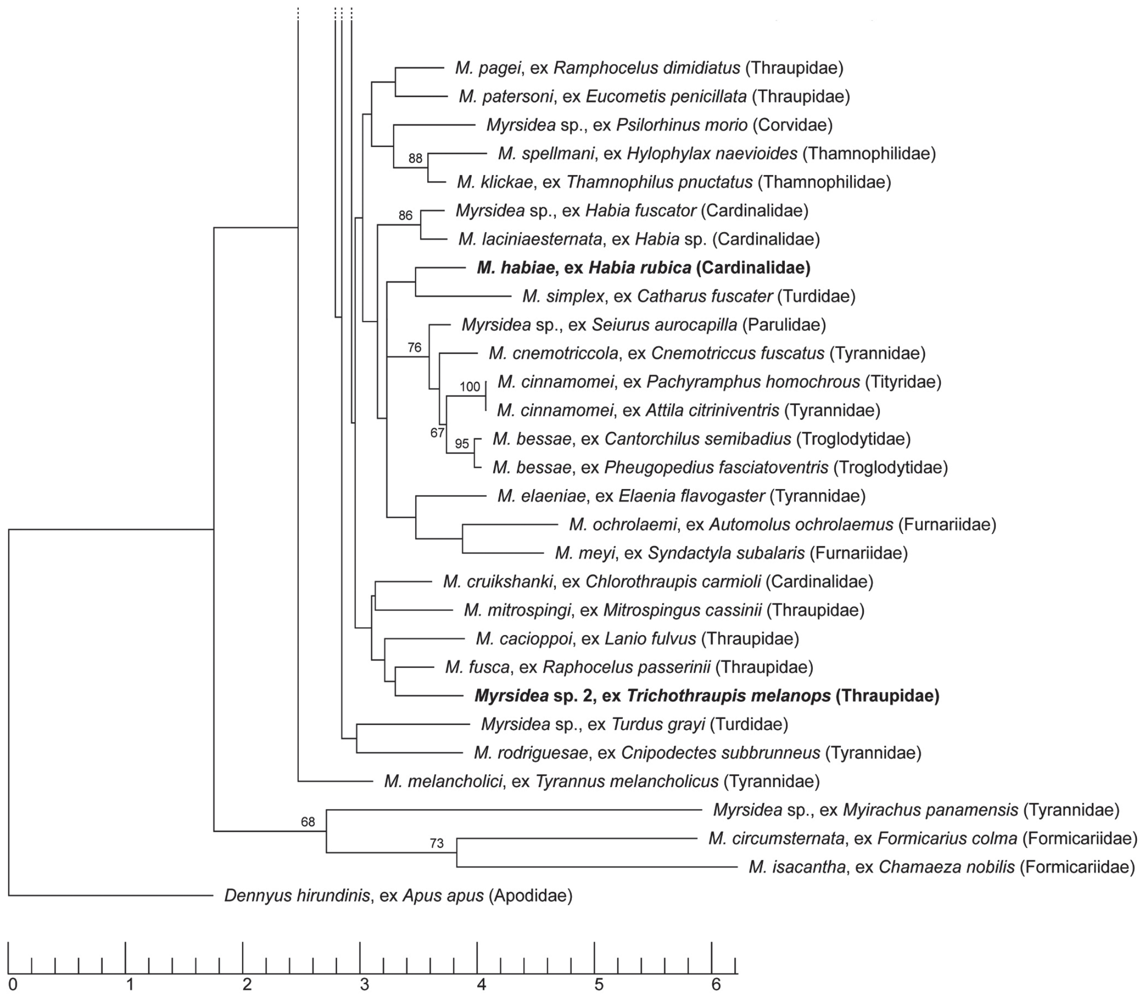


FIGURE 10. (Continued)

Interspecific genetic differences available from the literature also range from 12% to 27.3% with one exception: *Myrsidea goodmani* Price & Johnson, 2006a versus *Myrsidea batesi* Price & Johnson, 2006a, with p-distance 10.7%. Price & Johnson (2006a) mentioned that such a relatively low genetic difference, however surprising considering their morphological disparity, mirrors a similar genetic divergence between different populations of their hosts from different localities. Similar examples have been reported by Bueter *et al.* (2009) and Light *et al.* (2016), who also found genetic differences between *Myrsidea* species occurring on different populations of their hosts. Bueter *et al.* (2009) reported (1) *Myrsidea incerta* versus *Myrsidea pricei* Clay, 1966 with p-distance 7.4%, and with only minimal morphological differences, (see also Clay 1966), and (2) *Myrsidea incerta* versus *Myrsidea* sp. from *Seiurus aurocapillus* with p-distance 9.2%. Light *et al.* (2016) reported *Myrsidea* sp. from *Terpsiphone batesi* and *Terpsiphone rufiventer* with p-distance 5.7%, and *Myrsidea* sp. from different populations of *Alethe diademata* with p-distance 10.5%.

Our results from *M. lightae* fall well into this category: we found increasing genetic variability among *Myrsidea* from distant geographic areas—Honduras versus Panamá (8.2%), Panamá versus Paraguay (11.1%), and Honduras versus Paraguay (11.4%)—but only minimal morphological differences. In these cases, the concept of subspecies can be used, as Palma & Price (2010) applied it to two morphologically distinct populations of *Myrsidea nesomimi* from the Galápagos Islands, which were later confirmed by genetic data by Štefka *et al.* (2011). Štefka *et al.* (2011) reported that *M. nesomimi* from one locality or from a few close ones showed minimal genetic differences (0.1–0.6%), while lice of the two subspecies from different hosts and distant localities showed increasing genetic variability (4.5–5.1%).

According to available data on interspecific genetic diversity, we are confident that a clear and reliable limit for

distinguishing *Myrsidea* species within the genus and in particular among Neotropical *Myrsidea*, could be proposed at 12% divergence. This is in agreement with other authors who have described interspecific variability within *Myrsidea* (see Table 3 for references). The only way to resolve questionable cases is a combined approach, where morphology supports genetic data, and viceversa.

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